Bacterial aetiology of community-acquired pneumonia in hospitalised patients with chronic obstructive pulmonary disease in central Greece

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Community-acquired pneumonia (CAP) is characterised by inflammation and consolidation of lung tissue, followed by resolution accompanied by fever, chills, cough and difficulty in breathing, and is caused mainly by infection. The most commonly involved microorganisms are *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Chlamydia pneumoniae*, and *Legionella pneumophila* serogroup 1.

In patients with CAP, chronic obstructive pulmonary disease (COPD) is the most frequent respiratory co-morbidity in those aged over 65 years.[1] A common, preventable and treatable disease, COPD is characterised by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. It is found to be an independent factor related to severity in patients with CAP.[2]

Although, acute exacerbations of COPD due to CAP are very common, the pattern of antibiotic prescription varies widely from country to country, and there is no clear rationale for antibiotic choices. This is explained by the fact that COPD is associated with specific aetiologies such as *H. influenzae* and multidrug-resistant (MDR) pathogens.[3] Thus, the choice of the antibiotic should be based on the local bacterial epidemiology and the local bacterial resistance pattern.

Initial empirical treatment usually is an aminopenicillin with or without clavulanic acid, macrolide or tetracycline.[4,5] In patients with frequent exacerbations, culture of sputum or other material from the lung should be performed as Gram-negative bacteria (e.g., *Pseudomonas aeruginosa*) or resistant pathogens that are not sensitive to these antibiotics may be present.

In the present study, the prevalence of bacterial pathogens is determined among 56 hospitalised CAP patients with COPD in central Greece, between 2007 and 2009, in order to establish local guidelines for therapeutic options against CAP in COPD patients.

The patients were hospitalised in the Respiratory Department of the University Hospital of Larissa (UHL), the only 700-bed tertiary care hospital in Central Greece, covering an urban population of 600,000 inhabitants. Admission of the patients was based on the Patients Outcome Research Team (PORT) pneumonia severity index (PSI) and CURB-65 severity score system. Patients with severe immunosuppression (e.g., HIV infection, neutropenia) and cases of pneumonia occurring more than three days after hospitalisation were excluded.

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Bronchial secretions, urine and serum were obtained from all patients and were sent to the microbiological department of UHL. Bronchial specimens were cultured for common pathogens and also examined by molecular methods (i.e., polymerase chain reaction [PCR]) for the presence of DNA for *M. pneumoniae*, *C. pneumoniae*, *L. pneumophila*, *S. pneumoniae* and *H. influenzae*.[6] Briefly, all bronchial specimens collected were homogenised at 37°C with four volumes of 10% Sputolysin for 20 min and then inoculated on blood agar, MacConkey agar and chocolate agar supplemented with bacitracin.

After incubation (24–48 h), colonies corresponding to pathogenic microorganisms were identified to species level by Gram stain, colony morphology, growth requirement for X and V factors, and by the automated Vitek 2 system (bioMerieux, France). *H. influenzae* isolates were classified to type b using a commercial slide agglutination test (Pastorex, Bio-Rad). Genomic DNA for molecular assay was extracted from the remaining bronchial samples, as previously reported.[7] The molecular detection of the microorganisms was assessed by real-time PCR, as previously reported.[6]

Two serum specimens were collected, the first on admission and the second two weeks later; both were examined by immunofluorescence for the presence of IgM and IgG antibodies (Alphadia) against three atypical pathogens (*M. pneumoniae*, *C. pneumoniae*, *L. pneumophila*). In addition, urine samples were tested for the detection of *S. pneumoniae* and *L. pneumophila* antigens (Binax).

The aetiology was considered definite when one of the following criteria was met: i) bronchial culture yielded bacterial pathogen (in the absence of an apparent extrapulmonary focus), ii) single IgM titre for *C. pneumoniae* (≥32), *M. pneumoniae* (any positive titre) and *L. pneumophila* serogroup 1 (≥128), iii) seroconversion (i.e., a four-fold increase in IgG titre between the first and the second serum specimen), iv) positive urinary antigen test for *L. pneumophila* serogroup 1 and *S. pneumoniae*, and v) positive sputum PCR for any typical or atypical bacterium.

According to patient demographic data, the majority were male (48 male versus eight female) and mean age was 72±5 years. Forty-seven of the 56 hospitalised patients (84%) were found to be positive for CAP-related bacteria (Table 1). Typical pathogens were detected in 46/47 patients (98%), while atypical pathogens were found in only one patient (2%). The two most frequent pathogens were *H. influenzae* and *P. aeruginosa* (Table 1). No patient was found to be infected with two pathogens.

The best diagnostic method for *H. influenzae* was real-time PCR. Fourteen clinical specimens were found to be positive for this microorganism by culture and PCR, while an additional 22 clinical specimens were found to be positive by PCR alone (Table 1).

The results presented here are in accordance with several recent studies suggesting that Gram-negative species are usually isolated in CAP patients with COPD,[8,9] with *H. influenzae* remaining the most common isolate overall in these studies Therefore, although CAP patients are traditionally treated with antibiotics targeting Gram-positive or atypical bacteria, the present findings demonstrate the need to use antibiotics targeting Gram-negative bacteria,

Table 1. Microorganisms isolated from CAP patients with COPD and the methods used for detection.

		Diagnostic method			
	Number of patients	Culture	AD	IFA IgM/four-fold IgG	Real-time PCR
Streptococcus pneumoniae	3	3	3		3
Haemophilus influenzae type b	8	8			8
Haemophilus influenzae non-typeable	28	6			28
Pseudomonas aeuginosa	7	7			
Total typical pathogens	46	20	3		39
Mycoplasma pneumonia	1			1	
Chlamydia pneumonia				0	
Legionella pneumophila				0	
Total atypical pathogens	1			1	

AD: antigen detection IFA: immunofluorescence

especially *H. influenzae* and *P. aeruginosa*, in CAP patients with COPD.

These results demonstrate that molecular detection of *H. influenzae* in sputum of COPD patients with CAP is superior to conventional culture methods, and are in agreement with other previously reported studies.[10] Additionally, the report emphasises the value of conventional methods (culture and serological tests) in combination with molecular methods for the rapid and accurate identification of aetiological agents in COPD patients with CAP.

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